SHILAP Revta. lepid., 33 (131), 2005: 299-317 SRLPEF ISSN:0300-5267

Cladistic analysis of moon moths using morphology, molecules, and behaviour: *Actias* Leach, 1815; *Argema* Wallengren, 1858; *Graellsia* Grote, 1896 (Lepidoptera: Saturniidae)

J. Ylla, R. S. Peigler & A. Y. Kawahara

Abstract

A phylogenetic analysis of 16 moon moth species was conducted using morphology, molecules and behaviour. A data matrix of morphology and behaviour together was comprised of 93 characters from the larvae, pupae, cocoons, and adults in all ingroup species and two outgroups. Molecular data included 2662 nucleotides from elongation factor 1-alpha (EF 1-α) and dopa decarboxylase (DDC) protein coding nuclear genes of six ingroups and the two outgroups. Both data matrices were analyzed separately, compared, and combined. The total evidence analysis including all characters reveals the following generic relationships: (outgroups (*Argema* (*Graellsia* + *Actias*))). Character evolution indicates that the short hindwing tail evolved once and lengthened multiple times in different lineages of moon moths. Results support retaining *Graellsia* as a genus separate from *Actias*.
KEY WORDS: Lepidoptera, Saturniidae, *Actias*, *Argema*, *Graellsia*, cladogram, phylogeny, conifer feeding, tail length, total evidence analysis.

Análisis cladístico de las "mariposas luna" utilizando caracteres morfológicos, moleculares y etológicos: *Actias* Leach, 1815; *Argema* Wallengren, 1858; *Graellsia* Grote, 1896 (Lepidoptera: Saturniidae)

Resumen

Se presentan los resultados del análisis filogenético llevado a cabo con 16 especies de "mariposas luna" utilizando caracteres morfológicos, moleculares y etológicos. Los datos morfológicos y etológicos se han agrupado en una matriz que comprende un total de 93 caracteres obtenidos del estudio de las larvas, crisálidas, capullos y adultos pertenecientes a 14 especies "ingroup" y a 2 especies "outgroup". Los datos moleculares incluyen 2.662 nucleótidos de los genes que codifican el factor de elongación 1-alfa (EF 1-α) y la dopa descarboxilasa (DDC) pertenecientes a 6 de las especies "ingroup" y a las 2 "outgroup". Ambas matrices de datos han sido analizadas por separado, comparadas y combinadas. El análisis de evidencia total, obtenido agrupando todos los caracteres, revela la siguiente relación genérica: (outgroups (*Argema* (*Graellsia* + *Actias*))). Los resultados indican que la cola corta de las alas posteriores, una vez aparecida, evolucionó alargándose varias veces y de forma independiente en distintos linajes de "mariposas luna". Los resultados confirman la validez del género *Graellsia*, debiendo pues mantenerlo separado de *Actias*.
PALABRAS CLAVE: Lepidoptera, Saturniidae, *Actias*, *Argema*, *Graellsia*, cladograma, filogenia, longitud de las colas, análisis de evidencia total.

Introduction

The large moon moths in the genera *Actias* Leach, 1815, *Argema* Wallengren, 1858 and *Graellsia* Grote, 1896, with their tailed hindwings and delicate green, yellow and rose colouration are highly po-

pular with lepidopterists and arguably among the most beautiful insects in the world (CODY, 1996). The group includes about 30 species, distributed mainly in tropical and subtropical Asia, with fewer representatives in southwestern Europe (1), North and Central America (2), Madagascar (1), sub-Saharan Africa (3), and the eastern Palaearctic region (about 4). Larval hostplants of many species include resinous trees in families such as Pinaceae, Hamamelidaceae, Anacardiaceae, Juglandaceae, Myrtaceae, and Ebenaceae (PEIGLER, 1986).

A large amount of information has been published on the natural history of moon moths. JORDAN (1911), MOUCHA (1966), and CODY (1996) each depicted several species in colour paintings, and D'ABRERA (1998) showed virtually all of them as life-size images in colour photographs. ZHU & WANG (1996) provided more color photographs, hostplant information, and distributions of several species of *Actias*, and life history descriptions have been published for certain species in this genus: *Actias maenas* (NÄSSIG & PEIGLER, 1984), *A. groenendaeli* (PAUKSTADT & PAUKSTADT, 1993), *A. isis* (NAUMANN, 1995), *A. callandra* (MOHANRAJ *et al.*, 1996) and *A. ignescens* (VEENAKUMARI *et al.*, 2005). YLLA (1997) studied the natural history of *Graellsia isabelae* in detail, and a taxonomic treatment of the two American species of *Actias* was given by LEMAIRE (1978). Adults, larvae, genitalia, and cocoons for the genus *Argema* were illustrated by TESTOUT (1940), GRIVEAUD (1961), and PINHEY (1972). An excellent treatise containing many figures and morphological data on our two outgroup species, namely *Eudia pavonia* and *Saturnia pyri*, can be found in JOST *et al.* (2000), who also treated *Graellsia isabelae*. Incidentally, the drawings of male genitalia of *Eudia pavonia* from Europe shown in JOST *et al.* (2000) and from northern China by ZHU & WANG (1996) differ significantly. The ultrastructure of eggshells of a few moon moths plus our two outgroup species was studied by REGIER *et al*. (2005), who showed photographs of the chorions of *Saturnia pyri* and *Graellsia isabelae*.

Despite the aforementioned advances in our knowledge, a phylogenetic analysis including many species of moon moths has never been conducted. The only phylogenetic analysis to treat *Actias*, *Argema*, and *Graellsia* is the molecular study by REGIER *et al.* (2002), which included five species of moon moths. The purpose of this study is to use modern cladistic methodology to: (1) infer relationships of 16 species of moon moths using morphological, behavioural, and molecular data, (2) test the monophyly of *Actias* and *Argema*, and (3) determine whether *Graellsia* is better treated as a synonym of *Actias* or a separate genus. We selected a small but diverse sample of adults and larvae to illustrate here in colour (Figs. 1-6, 11-14).

Materials and Methods

Taxon sampling and dissection for morphological characters

Morphological data were recorded and coded by the first author from specimens reared by him or supplied by others. Data were obtained from the larvae, pupae, cocoons, and adults of 16 ingroups and two outgroups (Table 1). Each larva was preserved in 70% isopropyl alcohol. Adult genitalia were removed from dried, pinned specimens, boiled in a 10% solution of potassium hydroxide, and slide-mounted for study. Terminology for larval structures follows STEHR (1987), and for adult structures follows COMSTOCK (1918), SNODGRASS (1935), MATSUDA (1965, 1970, 1976), and KLOTS (1970).

We did not have larvae of six species available for study, but extracted larval characters of five from literature: *Actias callandra* (MOHANRAJ *et al.*, 1996), *A. groenendaeli* (PAUKSTADT & PAUKSTADT, 1993; PEIGLER & WANG, 1996), *A. ignescens* (VEENAKUMARI *et al.*, 2005), *A. isis* (NAUMANN, 1995), and *A. ningpoana* (HEPPNER *et al.*, 1998); larvae of *A. rhodopneuma* were not available nor have descriptions been published. We selected two species as outgroups, *Eudia pavonia* and *Saturnia pyri*, because multiple specimens of all life stages were available, and the clade containing them is possibly the sister-group to the moon moths (see REGIER *et al.*, 2002). These two outgroups were chosen for this study simply to root the trees.

Table 1. Species included in the present study and their geographical distributions

Actias artemis (Bremer & Gray 1853) - eastern Palaearctic, including Japan
Actias callandra Jordan 1911 - Andaman Islands, India
Actias dubernardi (Oberthür 1897) (Figs. 3, 5) - southern China
Actias groenendaeli Roepke 1954 - Lesser Sunda Islands, Indonesia
Actias ignescens Moore 1877 - Andaman Islands, India
Actias isis (Sonthonnax 1899) - Sulawesi Island, Indonesia
Actias luna (Linnaeus 1758) - eastern North America
Actias maenas Doubleday 1847 (= *leto*) (Fig. 2) - southeastern Asia
Actias ningpoana C. Felder & R. Felder 1862 - eastern China; Taiwan
Actias rhodopneuma Röber 1925 - southeastern Asia
Actias selene (Hübner "1806" [1807]) - China; Afghanistan to Java
Actias sinensis (Walker 1855) (= *heterogyna*) (Figs. 4, 6) - southeastern Asia
Actias truncatipennis (Sonthonnax 1899) (Fig. 1) – Mexico
Argema mimosae (Boidsduval 1847) (= *bouvieri*) (Fig. 11) - sub-Saharan Africa
Argema mittrei (Guérin-Méneville 1847) (Figs. 12-13) – Madagascar
Graellsia isabelae (Graells 1849) (Fig. 14) - southwestern Europe
Saturnia pyri [Denis & Schiffermüller 1775] - western Palaearctic
Eudia pavonia (Linnaeus 1758) - pan-Palaearctic

Phylogenetic analysis of morphological and behavioural data

MacClade version 4.05 (MADDISON & MADDISON, 2002) was used to construct a data matrix (Table 2), which was subsequently converted into nexus format. The nexus file was executed in PAUP* 4.0 version 10b (SWOFFORD, 2002) and analyzed on a Macintosh OS9 with a G4 dual GHz processor. A branch-and-bound search was conducted and the maximum number of trees was set at 1000, the initial upper bound was computed by stepwise addition, and all shortest trees were saved. The matrix was also analyzed in Winclada version 1.00.08 (NIXON, 2002) and spawned in NONA version 2.0 (GOLOBOFF, 1993) on a personal computer with a 650 MHz Pentium III processor. We conducted a heuristic search by implementing tree bisection and reconnection (TBR), setting the maximum number of trees to keep at 1000 (hold1000), running 1000 replications of random taxon addition (mult*1000), branch swapping (max*), and 100 starting trees per replicate (hold/100). Bootstrap (FELSENSTEIN, 1985), parsimony jackknife (FARRIS *et al.*, 1996), and Bremer support (BREMER, 1988, 1994) were calculated in NONA. We implemented the following commands to calculate bootstrap and jackknife values: number of replicates = 1000; mult*20, h/20, max trees = 100 (without TBR). Bremer support values were calculated using multiple commands (mult*25; max*; h 5000; sub 1; find*; h 10000; sub 2; find*, h150000; sub 3; find*, h 25000; sub 4; find*; h 30000; sub 5; find*; bs;). We include here a description of each morphological and behavioural character and character state coded (Table 3). All characters were weighted equally and coded as non-additive, except characters 53, 62, 71, 75, 80, and 89, which were additive. Abbreviations throughout our text are: BP = bootstrap; BS = Bremer support; JK = jackknife.

Table 2. Morphological and behavioural data matrix for the 16 ingroup and 2 outgroup taxa included in this study. ? = unavailable data; * = inapplicable data.

Character Number (10)	1	2	3	4	5	6	7	8	9
Character Number	12345678901234567890123456789012345678901234567890123456789012345678901234567890123								
Eudia pavonia	01010000001111000?000110101000001000?01100101200100010000000?0?1100000200101000201110000010								
Saturnia pyri	01011000110110020?000110101000001100?00110101200112110010000?0?1100001200101010111110000010								
Graellsia isabelae	01010111111121021111120001010012011011001101110100112100 1*01110110103113001001011110001000000								

Argema mimosae	200100000012000100?0001??100110201?????100100010100 2?0111111020100102113021001000????????????
Argema mittrei	??????????????????0?1?000110100110 2????????????????????????????00113010002211200010000 1????????????
Actias artemis	201001010010000100000000110110010100111100010000110123011 1*011111101011110111010110 1*100110201
Actias callandra	11????11001001?100?0??011?11?0?1?001?1?????????????????????1110111101010?????????????????????2?1
Actias dubernardi	20??????0?1?0???11???????10100100001110101102?0110023011?00103100201101102110111101110100100*??
Actias groenendaeli	20?0??000112002?00?0??01?1??0?1100?????2011???????02?1101?1102110101101110110101 1?????????10?
Actias ignescens	201???000111001100?0????1?101101?????????????????????????????00103110101201?????????????????????01
Actias isis	20?0??000111002?001?1?????1011010?0?????20110??101002?0101011?3110101101301101121 1????????????
Actias luna	101000000010001000*000*101100110101111000100001100230111 1*101110010211111110101001111010201
Actias maenas	201001000111000000000?0111011010100010120110001010023010100103110101201301211112101110012020 1
Actias ningpoana	201000010010010100?????110111011110111111?????00?????2301111010111101011?????????????????????2?1
Actias rhodopneuma	??????????????????????????????????010?????00111??101012?1101001?21001011?0101211101 1????????????
Actias selene	201100010010010100?000011011101110011112001000101012301111110111101011220111010110011001 21*?1
Actias sinensis	201101000010001000000001111110101101111200100010100220111101011100101021002001011010111121201
Actias truncatipennis	101000000010001000?00101?011001111011110011000101002311 1?1101110010211111110101101111012 0*??

Table 3. Character and character-state descriptions for the taxa included in this study. Characters are arranged by life stage and body region, beginning with the head. "Larva" refers to the fifth instar unless otherwise stated.

Larval characters:

1. Predominant colour of first-instar larva: 0= black; 1= green; 2= orange.
2. Predominant colour of second and third instar larva: 0= greenish-orange; 1= dark brown.
3. Two-segmented antenna of larva: 0= both segments equal in length; 1= pedicel twice the length of scape.
4. Triangular shape of larval frons: 0= equilateral; 1= isosceles.
5. Larval mandibles: 0= without visible teeth, margins smooth; 1= with four distinct teeth.
6. Lateral expansions of spinneret: 0= absent; 1= present.
7. Colour of thoracic legs of larva: 0= black or brownish; 1= reddish.
8. Scoli on thorax compared to scoli on abdominal segments 1-7 of larva: 0= equal in size; 1= longer.
9. Length of thoracic setae compared to scoli on abdominal segments 1-7: 0= equal in length; 1= longer.
10. Cuticular longitudinal folds below lateral scoli: 0= absent; 1= present.
11. Terminal shape of long setae on scoli: 0= club-shaped; 1= finely pointed.
12. Subdorsal scoli: 0= somewhat more simple than dorsal scoli, often transformed into chalazae; 1= appearing like dorsal scoli; 2= reduced to single seta or absent.
13. Dorsal scoli of eighth abdominal segment of larva: 0= fused into one; 1= separate as on other body segments; 2= one partially fused double scolus.
14. Anal plate on tenth abdominal segment of larva: 0= without two lateral scoli; 1= with two lateral scoli.
15. Hairs covering body of larva: 0= elongate; 1= shortened.
16. Banding on larva: 0= without bands or lines; 1= with bands and/or lines.
17. Short cuticular setae of larva (those that do not exhibit a defined structure such as scoli, chalazae, verrucae, or verricle): 0= all thick; 1= some slender; 2= all slender.
18. White spots on cuticular surface of larva: 0= absent; 1= present.
19. Larval fluid secretion when disturbed: 0= absent; 1= present.

Pupal and cocoon characters:

20. Colour of pupa: 0= black, dark brown, or brown; 1= reddish.
21. Length of antennal imprint compared to length of pupa in male: 0= approximately one-third; 1= approximately half.
22. Contact of internal margin of antennal imprint on male pupa: 0= separated; 1= in contact with less than half of antennal length at apex; 2= internal margin in contact with at least half of antennal length.
23. Arrangement of hooks on cremaster: 0= circular; 1= linear.
24. Pupal location: 0= on the ground; 1= on the hostplant.
25. Pupal behaviour when disturbed: 0= inactive or only slightly moving; 1= very active.
26. Ecdysial suture of cocoon: 0= absent; 1= present.
27. Impression of substrate on cocoon: 0= absent; 1= present.
28. Filaments on cocoon: 0= absent; 1= present.

29. Perforations on cocoon: 0= absent; 1= present.
30. Peduncle on cocoon: 0= absent; 1= present.
31. Strength of cocoon: 0= strong, resistant to pressure; 1= papery and flimsy.
32. Male antennal width (at widest point): 0= less than 4.5 mm; 1= greater than 5 mm.

Adult characters:

33. Bipectinate segments on tip of male antenna: 0= distal 3-5 segments; 1= distal 6-8 segments.
34. Length of antenna compared to length of thorax in male: 0= equal or slightly shorter; 1= distinctly longer.
35. Length of antenna compared to length of thorax in female: 0= equal or slightly shorter; 1= distinctly longer.
36. Rami length of female antenna: 0= reduced; 1= elongate.
37. Pectination of female antenna: 0= bipectinate; 1= quadripectinate.
38. Length of basal rami compared to length of apical rami at widest point female antenna: 0= slightly longer; 1= greater than twice the length.
39. Length of rami compared to length of an antennal segment in female: 0= 1-2.5 times length of segment; 1= more than 2.5 times length of segment.
40. Hump on frons: 0= absent; 1= with a central hump; 2= with two lateral humps beside eyes.
41. Frontoclypeal suture: 0= smooth or wavy, without dentitions; 1= with lateral teeth.
42. Transclypeal band: 0= weakly or barely visible; 1= well defined.
43. Hair covering frons: 0= sparse and erect; 1=dense and flattened.
44. Hair colour of frons: 0= yellow; brown.
45. Labrum covering mandible: 0= half; 1= entire.
46. Division of labrum by central notch: 0= into equal halves; 1= into unequal halves.
47. Shape of labial palpus: 0= straight; 1= curved; 2= bulbous.
48. Length of labial palpus: 0= short; 1= long.
49. Segmentation of labial palpus: 0= one-segmented; 1= two-segmented.
50. Hair colour of labial palpus: 0= reddish purple; 1= brown.
51. Maxillary palpus: 0= absent or barely visible; 1= large and clearly visible.
52. Male tibial epiphysis: 0= absent; 1= present, length less than apical third of tibia; 2= present, length greater than apical third of tibia.
53. Female tibial epiphysis: 0= absent; 1= very short; 2= approximately length of midpoint of tibia; 3= very long.
54. Margin of tibial spurs on meso- and metathoracic legs of male: 0= serrate; 1= smooth.
55. Shape of tibial spurs on meso- and metathoracic legs of male: 0= needle-shaped; 1= spoon-shaped.
56. Scale colour of femur in relation to scale colour of tibia and tarus: 0= identical; 1= lighter.
57. Male pulvillus: 0= absent; 1= present.
58. Number of forewing radial veins: 0= four; 1= five.
59. Shape of outer margin of forewing in male: 0= straight; 1= distinctly concave.
60. Tails on hindwings of both sexes: 0= absent; 1= present.
61. Length of male hindwing tail to that of female: 0= equal; 2= distinctly longer.
62. Relation of tail length to forewing costal length in male: 0= <75%; 1= 90–110%; 2= 130–160%.
63. Position of hindwings tails in repose: 0= crossed; 1= parallel.
64. Eyespot on hindwing of male: 0= absent; 1= present.
65. Eyespot on forewing of male: 0= connected to costa; 1= separate from costa.
66. Shape of eyespot: 0= round or oval in both forewing and hindwing; 1= crescent-shaped in forewing but rounded in hindwing; 2= oval in forewing but obsolete (absent or nearly so) in hindwing.
67. Hyaline of eyespot: 0= absent; 1= present.
68. Diameter of eyespot in hindwing of both sexes: 0= less than distance of M1-M2; 1= greater than distance of M1-M2.
69. Red scales along wing veins: 0= absent; 1= along forewing costa; 2= along costa and wing margins; 3= along majority of veins in both wings.
70. Sexual dimorphism of wings: 0= dimorphic; 1= sexes alike.
71. Lobes on bifid uncus: 0= reduced; 1= with two dorsal lobes; 2= with two dorsal lobes and two frontal lobes; 3= with two frontal lobes.
72. Gnathos: 0= reduced; 1= collar-shaped, without a basal process; 2= collar-shaped, with two clear basal processes; 3= spatula-shaped, without a basal process.
73. Sclerotization of transtilla: 0= weak; 1= heavy.

74. Juxtal process: 0= absent; 1= symmetrical; 2= asymmetrical.
75. Length of aedeagus compared to distance from uncus to saccus: 0= less than 2/5; 1= between 1/2 and 2/3; 2= greater than 4/5.
76. Anellus: 0= absent; 1= present.
77. Saccus: 0= reduced; 1= enlarged.
78. Valva: 0= simple; 1= bilobed.
79. Membranous expansions on inner surface of valva: 0= absent; 1= present.
80. Number of distal processes of sacculus: 0= zero; 1= one; 2= two.
81. Penicullum: 0= absent; 1= present.
82. Length of anterior apophysis compared to length of bursa copulatrix: 0= very short; 1= approximately equal.
83. Shape of lamella postvaginalis: 0= spatula-shaped; 1= spoon-shaped.
84. Antrum: 0= reduced; 1= enlarged.
85. Length of ductus bursae: 0= short; 1= long.
86. Width of ductus bursae: 0= narrow; 1= thick.
87. Junction between ductus bursae and ductus seminalis: 0= at base of ductus bursae; 1= middle of ductus bursae.
88. Corpus bursae: 0= reduced; 1= enlarged.
89. Number of signa on corpus bursae: 0= zero; 1= one; 2= two.
90. Fissure of ostium bursae: 0= transverse; 1= rounded.

Behavioural characters:
91. Generations: 0= univoltine; 1= bivoltine; 2= multivoltine.
92. Period of adult activity: 0= nocturnal; 1= diurnal.
93. Diapause: 0= obligate; 1= facultative.

Phylogenetic analysis of molecular data

The combined molecular dataset of elongation factor 1-alpha (EF 1-α) and dopa decarboxylase (DDC)from REGIER *et al.* (2002) was reexamined in PAUP*. Five species from the dataset were included. EF-1-α sequences of *A. artemis*, *E. pavonia*, and *S. pyri* were provided by J. Regier (Genbank Accession Numbers: DQ077814, DQ077815, DQ077816) (Table 4). The molecular parsimony analysis included 2569 characters combined from EF1-α and DDC. The analysis was conducted in PAUP*, and *E. pavonia* and *S. pyri* were defined as outgroups. A heuristic search was conducted by implementing TBR with 1000 maximum trees, 1000 replicates, 100 trees held at each step, and only the best trees were kept. A heuristic search was also conducted retaining groups with >50% frequency, random sequence addition, TBR, 1000 replicates, and 20 trees held. All characters were weighted equally.

Congruence between morphological and molecular topologies

To assess congruence between the morphological and molecular tree, we constrained the morphological analysis to match the molecular topology. We also constrained the molecular analysis to fit the morphological topology. Constraints were implemented by comparing the relationships of the eight species in which both morphology and molecules were available (Table 4). The constrained and unconstrained topologies for each dataset were compared using the "constraints = (backbone)" command in PAUP*, and tested for significance by employing the Kishino-Hasegawa (KISHINO & HASEGAWA, 1989) and Templeton (TEMPLETON, 1983) tests.

Total evidence analysis

A total evidence analysis was conducted by combining morphological and behavioural data with the subset of molecular data from REGIER *et al*. (2002). Although the available molecular data were limited to eight taxa, we combined morphological and molecular data because the two data types independently gave different topologies (see Results). The matrices were combined and a heuristic search was conducted in PAUP* by implementing TBR with 1000 maximum trees, random addition with 1000

replicates, 100 trees held at each step, and only the best trees were kept. Analyses were initially conducted with many different outgroups in a variety of different combinations, but all except two outgroups were removed in the final analysis because outgroups did not change relationships of ingroup taxa, only adding many trees, each differing slightly in outgroup relationships. Adding outgroups with missing morphological data resulted in many trees because *Argema mittrei* had very few data coded, and this taxon placed anywhere among outgroups.

Table 4. Data types analyzed in this study. "+" denotes complete data; "-" indicates data in which the majority (> 50%) of the characters were coded, but some were missing. EF1-α sequences are each 1240 nucleotides in length, DDC sequences are 1051 nucleotides in length. Refer to REGIER *et al.* (2002) for GenBank accession numbers for all taxa, except those with sequence data new to this report (*). Ingroups listed first, the two outgroups listed thereafter.

Taxon	Morphology			Behaviour	Molecules	
	Larva	Pupa	Adult		EF1-α	DDC
*Saturnia pyri**	+	+	+	+	+	
*Eudia pavonia**	+	+	+	+	+	
*Actias artemis**	+	+	+	+	+	
Actias callandra	+	+	-	+		
Actias dubernardi	-	+	+	-		
Actias groenendaeli	+	+	+	+		
Actias ignescens	+	+	-	-		
Actias isis	+	+	+	+	+	+
Actias luna	+	+	+	+	+	+
Actias maenas	+	+	+	+		
Actias ningpoana	+	+	-	+		
Actias rhodopneuma	-	-	+	-		
Actias selene	+	+	+	+	+	+
Actias sinensis	+	+	+	+		
Actias truncatipennis	+	+	+	-		
Argema mimosae	+	+	+	-	+	+
Argema mittrei	-	+	-	-		
Graellsia isabelae	+	+	+	+	+	+

Results

Phylogenetic analysis of morphological and behavioural data

The morphological analysis resulted in a single most parsimonious tree (Fig. 7) with a length of 225 steps (CI = 0.52, RI = 0.61), with the following generic relationships: (outgroups (*Graellsia* (*Argema* + *Actias*))). PAUP* and NONA both generated the same tree. There were 84 parsimony informative characters, and six unambiguous synapomorphies supported the monophyly of *Actias* + *Argema* + *Graellsia* (character numbers are shown in parentheses after descriptions throughout the text): quadripectinate female antenna (37); reddish purple hairs on labial palpus (50); presence of tails on hindwings of both sexes (60); two dorsal lobes on uncus (71); length of aedeagus between one-half and two-thirds the distance between the uncus and saccus (75); and transverse fissure of ostium bursae (90). There were twelve synapomorphies supporting the sister-group relationship of *Argema* + *Actias*, and three supporting the monophyly of *Actias*: length of antenna slightly shorter or equal to length of thorax in male (34); parallel position of hindwing tails held in repose (63); and collar-shaped gnathos without a basal process (72). Clade support for *Actias* + *Argema* + *Graellsia* was relatively high (BP = 96%, JK = 83%, BS = 3), but all three other clades had BP and JK support values less than 50%. The topology did not change when all characters were coded as non-additive (L = 223 steps, CI = 0.52, RI = 0.60).

Phylogenetic analysis of molecular data

The molecular analysis resulted in one most parsimonious tree (Fig. 8) with a length of 402 steps (CI = 0.88, RI = 0.71). There were 129 parsimony informative characters. Support was high (BP 80%) for all nodes. When the number of outgroups was changed, the relationships of the ingroups did not change. Relationships of the three ingroup genera were identical to the results of REGIER *et al*. (2002): (outgroup (*Argema* (*Graellsia* + *Actias*))).

Congruence between morphological and molecular topologies

When the morphological data were constrained to the molecular topology (*Argema mimosae* (*Graellsia isabelae* (*Actias luna* (*A. isis* (*A. artemis* + *A. selene*))))) and analyzed, 10 most parsimonious trees were obtained (L = 230, CI = 0.51, RI = 0.59). When compared to the unconstrained tree, each of the 10 trees demonstrated that morphology did not strongly prefer the morphological topology over the molecular topology (KH/Templeton: $0.411 < P < 0.551$). When the molecular data were constrained to the morphological topology (*Graellsia isabelae* (*Argema mimosae* (*Actias isis* (*A. luna* (*A. artemis* + *A. selene*))))), a single most parsimonious tree was obtained (L = 410, CI = 0.86, RI = 0.66) and the molecular data reject the morphological topology with strong statistical significance (KH: $P = 0.0209$, Templeton: $P = 0.0386$).

Total evidence analysis

The total evidence analysis resulted in six most parsimonious trees (L = 636, CI = 0.74, RI = 0.62), and the strict consensus cladogram (Fig. 9) reveals relationships congruent with the molecular tree. All six trees were similar in topology, differing slightly in the sister-group relationships of *Actias dubernardi*, *A. groenendaeli*, and *A. rhodopneuma*. Three trees also resulted in a paraphyletic *Argema*. We investigated all topologies, and examined the evolution of tail length on one (Fig. 10). Character state changes in tail length that are present in all six topologies are indicated on this tree, along with one parallelism (homoplasy) which is not present in all trees. We chose this topology for two reasons: (1) because the morphological analysis alone placed *A. groenendaeli* and *A. rhodopneuma* as sister species (Fig. 7), and (2) because the paraphyly of *Argema* was clearly an artifact of *A. mimosae* having molecular characters which were missing for *A. mittrei*.

Discussion

Monophyly and composition of *Actias, Argema* and *Graellsia*

Morphology and molecules both strongly support the monophyly of *Actias* + *Argema* + *Graellsia*. However, the morphological analysis resulted in a sister-group relationship of *Actias* + *Argema* (BP/JK = 76%, BS = 3) that differs from our molecular analysis and that of REGIER *et al.* (2002), both of which grouped *Actias* and *Graellsia* as sister genera (BP = 98% and 99%, respectively). There appeared to be minimal conflict between the two genes sampled in both molecular analyses, as support for this clade was very high in molecular analyses. Statistical comparisons indicate that there is stronger signal in the molecular data because molecules strongly reject the morphological topology, while morphology did not significantly prefer one over the other. The total evidence phylogeny also resulted in generic relationships that matched the molecular trees, although support was weaker (BP = 64%).

Monophyly of *Actias* is supported by three morphological synapomorphies, but clade support was low in the morphological analysis (BP, JK < 50%). The molecular parsimony analysis from our study and REGIER *et al.* (2002) also resulted in a monophyletic *Actias*, but in both cases, support values were lower than values obtained for *Graellsia* + *Actias* + *Argema* (BP = 80%, 68% respectively). The total

evidence analysis (Fig. 9) also recovers a monophyletic *Actias*, with *Actias sinensis* basal to the rest of the species in the genus (BP = 76%).

Argema is monophyletic in the morphological analysis, but weakly supported (BP = 54%, JK = 58%). Monophyly in this clade is lost in the strict consensus of the combined analysis, but was recovered when calculating bootstrap values (Fig. 10).

Phylogenetic position of *Graellsia*

Graellsia was synonymized with *Actias* by NÄSSIG (1991) because he believed that *Actias* would become paraphyletic. The evidence he provided was based on general similarity of the adult, such as the green, short-tailed *Graellsia* resembling *A. luna* more than the long-tailed, yellow and brown *A. isis*. Neither the morphological nor the molecular analysis confirm this synonymy. Taking into account only the topology of the morphological results, if *Graellsia* were synonymized with *Actias*, *Actias* should become paraphyletic with respect to *Argema* (consequently, *Argema* would also have to be synonymized with *Actias* to retain the monophyly of *Actias*). In the same way, our total evidence analysis also supports the interpretation of YLLA & SARTO (1993) and REGIER *et al*. (2002), becoming clear that this synonymy is not necessary. Although the category of genus is always subjective, we support the traditional classification where *Graellsia* is maintained as a separate genus.

Some might argue that hybridization is evidence for inclusion of *Graellsia* within *Actias*. Since 1979, a team of lepidopterists in France has hybridized *Graellsia isabelae* with *Actias artemis*, *A. luna, A. truncatipennis, A. sinensis, A. isis*, and *A. selene* (see ADÈS *et al.* 1995, and references cited therein). Most of the offspring of these crosses did not survive, but some developed into adults. Hybridization does not indicate that two genera should be synonymized (PEIGLER, 1978).

Evolution of the hindwing tails

Our results indicate that tail length evolved once from none (*Eudia* + *Saturnia*) to short. Tail length increased independently at least twice: once in the common ancestor of *Argema* and another time in *Actias* (Fig. 10). Very long tails (with a 130–160% forewing costa to tail length ratio, character 62, state 3) evolved independently in three different lineages, namely *Argema mittrei, Actias dubernardi*, and *Actias isis* + *A. ignescens* + *A. maenas*.

It is not surprising that very long tails would evolve more than once in the moon moths, because other saturniids in the Neotropical *Copiopteryx* Duncan, 1841 (Arsenurinae) and the African *Eustera* Duncan, 1841 (Saturniinae: Urotini) also have very long and slender tails. Such tails also occur outside the Saturniidae, such as in *Himantopterus* (Zygaenoidea: Himantopteridae), and even outside the Lepidoptera, such as in *Nemoptera* (Neuroptera: Nemopteridae). These tails must impart selective advantage. JANZEN (1984) wrote, "*Copiopteryx semiramis* flies slowly to fast in a nearly straight trajectory with a moderately rapid wing beat, and the long tails stream out behind with the tip of each tracing a five to 10 cm diameter circle in a plane at right angles to the trajectory of the moth. I suspect that the tails render this moth, the smallest (lightest) of the arsenurine saturniids at Santa Rosa, the largest saturniid in the Park in the sonar imagery of a bat."

The posture at which hindwing tails are held (character 63) evolved from an ancestral state in which tails were crossed (*Argema* and *Graellsia*) before becoming held parallel (Fig. 10). This characteristic was noted previously for *Actias* and *Argema* (NÄSSIG & PEIGLER, 1984; D'ABRERA, 1998), and we note here that *Graellsia* has a tendency to cross its tails when at rest, especially in females. *Actias angulocaudata* Naumann & Bouyer, 1998, has tails that appear crossed when they are held parallel. Parallel tails is a synapomorphy for all species of *Actias* included in this study.

Evolution of hostplant feeding

Our phylogenetic analysis indicates two independent origins for larval hostplant feeding on coni-

fers (Fig. 10). The first was along the *Graellsia* lineage, while the second came along the *Actias dubernardi* branch. These two montane taxa may have been forced to shift to conifers during Pleistocene glaciation. Central and western China is a region that harbours many relict species, such as *A. dubernardi*, and the Iberian Peninsula was a refugium during the Pleistocene (FERNÁNDEZ-VIDAL, 1992). Long known to feed on *Pinus* (MELL, 1950), *A. dubernardi* was recently found to accept other conifers such as *Cedrus deodara* (native to the Himalayas) and *Metasequoia glyptostroboides* (itself a central Chinese relic) in Texas. *Graellsia* is also known to accept conifers besides *Pinus*; results of hostplant trials were reported by YLLA (1997) and NÄSSIG (1991), who found that *Larix* was also a suitable food for *G. isabelae*. Phylogenetically, *Cedrus* and *Larix* are more closely related to *Pinus* than to other Pinaceae (e.g., *Abies, Picea, Tsuga*) (PHILLIPS & RIX, 2002), and *Cedrus* is also a suitable food for *Graellsia*. Since all other taxa in our analysis feed on angiosperms, two independent origins for conifer feeding is the most parsimonious hypothesis.

Biogeography and relationships of taxa, including ones not included in our analyses

Although several species were not included in our study because material was unavailable or insufficient, we can hypothesize their placement based on general appearance of the adult moths. Among African *Argema*, the yellow *A. kuhnei* Pinhey, 1969, from south-central Africa, should be the sister-species to the widespread, green *A. mimosae*, and this pair the sister-group to *A. besanti* Rebel, 1895; that clade of three mainland species is expected to be the extant sister-group to the Malagasy *A. mittrei*. *Actias angulocaudata* from central China appears very similar to *A. sinensis* and may be its sister-species. The Vietnamese *Actias chapae* Mell, 1950, is probably the sister-species of *A. dubernardi*, because of the shared unique wingshape; *A. chapae* was recently found in northern Guangdong, sympatric with *A. dubernardi* (MORISHITA & KISHIDA, 2000). The Japanese *A. gnoma* (Butler, 1877) and Chinese *A. felicis* (Oberthür, 1896) probably belong within the clade containing *A. selene* and *A. artemis*.

We had available a single pinned male of the Taiwanese *Actias neidhoeferi* Ong & Yu, 1968, and Ylla dissected the genitalia and tabulated the adult morphology. However, with the unavailability of a female specimen and no information on the immature stages, this species was eventually excluded from the present study, because initial PAUP* analyses made in Texas placed this species outside the genus *Actias*, and no better results were obtained in Maryland with the inadequate dataset. The intrageneric relationship of this species would be of particular interest, because it appears to be another montane relic with no obvious close relatives. We consider the name *Actias kongjiaria* Zhu & Wang, 1993, described from Sichuan, to be a synonym of *A. neidhoeferi*. This distribution on Taiwan and mainland China exactly corresponds to that of the relict species *Samia watsoni* (Oberthür, 1914) (PEIGLER & NAUMANN, 2003).

Our newly proposed phylogeny permits us to offer some hypotheses pertaining to the biogeography of the group. We believe that because *Argema* shows some plesiomorphic traits such as wide antennae (34, 35) and a typical saturniine cocoon (29, 30, 31), that it is probably more basal, and that a trans-Arabian dispersal (see HOLLOWAY & NIELSEN, 1999) from Africa to Asia and Europe gave rise to the other two genera of moon moths. This model parallels the conclusions of PEIGLER & NAUMANN (2003) for the biogeographical origin of the saturniid genus *Samia* Hübner, [1819]; those authors proposed that *Samia* evolved from an ancestor closer to the more basal African *Epiphora* Wallengren, 1860, dispersed to, and then radiated in eastern Asia. We note that *Argema besanti*, the smallest and northeasternmost representative of its genus in Africa, has strongly marked wing veins, as does *Graellsia*. The ancestor of *Graellsia* probably reached the Iberian Peninsula through southeastern Europe (FERNÁNDEZ-VIDAL, 1992). The eastern Himalayas have clearly been a centre of divergence for the genus *Actias*, giving rise to freeze-tolerant species in the eastern Palaearctic, and to additional tropical species that dispersed into Indonesia and the Philippines, leading to further vicariance. The two American species clearly share a common ancestor that dispersed from northeastern Asia across Beringia only comparatively recently, probably during the Pliocene or later. This pair (*A. luna*

and *A. truncatipennis*) has seasonal adult forms, as does the sister-taxon *A. sinensis*. While most of the aforementioned relationships are hypothetical, our comments will allow interested readers to construct a more complete cladogram for the entire group.

Sexual dimorphism (70) is the norm for the genus *Actias*, with females weakly marked and light green, and males more intensely marked in yellow, brown, or rose. Based on our hypothetical phylogeny, sexual dimorphism may have been lost in *A. selene*, and possibly never existed in the ancestor of the two American species. A reduction of antennae (34) is also apparent in the clades containing *A. selene* and *A. maenas* and their nearest relatives, whereas larger antennae can be seen in *A. luna, A. groenendaeli*, and *A. neidhoeferi*, and even larger ones in *Graellsia* and especially *Argema*.

Our results support the close relationship between several Asian species that have been considered conspecific. *Actias isis* and *A. ignescens* have been considered to be subspecies of *A. maenas* by some authors. *Actias callandra* and *A. ningpoana* have been treated as subspecies of *A. selene* by many authors. On the other hand, although *A. groenendaeli* was originally described as a subspecies of *A. maenas*, our study indicates that these two taxa are not so closely related. We believe that the eastern Indonesian distribution of *A. groenendaeli* suggests a more ancient dispersal event, like that proposed for one of the two species of *Samia* occuring on Sulawesi (PEIGLER & NAUMANN, 2003). Our result which proposes that the closest extant relative of *A. groenendaeli* might be *A. rhodopneuma* is therefore quite plausible.

Conclusion

Our study is the next step towards an understanding the relationships of moon moths. We conducted our analyses using all data available to us, but it is evident from the lack of full resolution and low support values for certain clades in the total evidence analysis that our knowledge is still incomplete. We would specifically suggest coding morphological characters for other Saturniinae outgroups that were included in the molecular analysis of REGIER *et al.* (2002), and sequencing EF1-α and DDC for the ten ingroup taxa for which there were no molecular data. More morphological and molecular data will be needed to test our hypotheses, enrich the matrix presented in this current study, and may shed light on reasons why topologies of moon moths differ between morphology and molecular data.

We cannot conclude our discussion without conveying our concern that many of these exquisite moths will become rare or even extinct during the twenty-first century, mainly due to deforestation which is occuring in most parts of their ranges. *Actias luna* is so widespread and common, that its future seems secure in the foreseeable future, but *A. truncatipennis* in Mexico is losing a lot of habitat to logging, and has a much more restricted distribution. *Actias artemis* in the Far East of Russia inhabits huge expanses of boreal forests, and in Japan, central Taiwan, and on the Andamans, some national parks have been established to preserve some of the remaining patches of habitats. Although generally safe from logging, these areas may decline due to acid rain and global warming. The large-scale introduction of eucalypt trees (*Eucalyptus*) onto Madagascar may actually provide a good source of food and habitat for *Argema mittrei*, as noted by D'ABRERA (1998), although Madagascar in general has badly managed its biological resources. In Spain, although it is not an endangered species, the frequent forest fires in summer and aerial applications of insecticides to combat forest pests (especially *Thaumetopoea pityocampa* [Denis & Schiffermüller 1775]) are detrimental to populations of *Graellsia isabelae* (YLLA, 1995). Deforestation in sub-Saharan Africa and all over tropical Asia is well-documented, but we acknowledge that consumers of wood products in the United States, Japan, and Europe have been major contributors to that habitat alteration. Deforestation leads to severe flooding in nearby areas, further harming habitats. Ecotourism in Indonesia, East Africa, the Philippines, Malaysia, Nepal, and other places offers some hope that individual governments will increasingly try to protect large areas of rainforest where moon moths exist. The authors consider themselves fortunate to have been able to rear and collect many of these moon moths, and hope that lepidopterists a century from now will have the same opportunities.

Acknowledgments

We thank the following for supplying valuable specimens used in this study: Prashanth Mohanraj and K. Veenakumari (Project Directorate Biological Control, Bangalore, India), Wolfgang A. Nässig (Senckenberg-Museum, Frankfurt am Main, Germany), Stefan Naumann (Berlin, Germany), Rolf G. Oberprieler (CSIRO, Canberra, Australia), Ulrich Paukstadt and Laela H. Paukstadt (Wilhelmshaven, Germany), Rikio Sato (Niigata, Japan), and Kirby L. Wolfe (California, USA). Víctor Sarto i Monteys (Universidad Autónoma de Barcelona, Spain) and Albert Masó i Planas (Universidad de Barcelona, Spain) deserve commendation for their long-term support of this study. Peigler's student Rowdy Cole Zett (University of the Incarnate Word, USA) assisted in data entry. Charles Mitter (University of Maryland, USA) and Jerome Regier (University of Maryland Biotechnology Institute, USA) provided unpublished EF1-α sequences of *S. pyri, E. pavonia*, and *A. artemis*, and gave critical comments on earlier versions of the manuscript, which improved its quality. Stephanie Johnson (University of Maryland, USA) also gave helpful comments on the manuscript, and Jeffrey Shultz (University of Maryland, USA) provided assistance on combining datasets. This study was funded in part by the National Science Foundation (DEB-0212910).

BIBLIOGRAPHY

ADÈS, D., COCAULT, R., LEMAÎTRE, R. & VUATTOUX, R., 1995.– Hybridation entre *Graellsia isabellae* [*sic*] ♂ et *Actias artemis* ♀ un succès...un sauvetage (Lepidoptera Saturniidae).– *Lambillionea,* **45**(1): 26-30.

BREMER, K., 1988.– The limits of amino acid sequence data in angiosperm phylogenetic reconstruction.– *Evolution,* **42**: 795-803.

BREMER, K., 1994.– Branch support and tree stability.– *Cladistics,* **10**: 295-304.

CODY, J., 1996.– *Wings of Paradise: the Great Saturniid Moths*: xix + 163 pp. University of North Carolina Press, Chapel Hill.

COMSTOCK, J. H., 1918.– *The Wings of Insects: An Exposition of the Uniform Terminology of the Wing-veins of Insects and a Discussion of the More General Characteristics of the Wings of the Several Orders of Insects*: xviii + 430 pp. Comstock, Ithaca, New York.

D'ABRERA, B., 1998.– *Saturniidae Mundi: Saturniid Moths of the World, part 3*: 171 p. Goecke & Evers, Keltern, Germany; Hill House, Melbourne & London.

FARRIS, J. S., ALBERT, V. A., KÄLLERSJÖ, M., LIPSCOMB, D. & KLUGE, A. G., 1996.– Parsimony jackknifing outperforms neighbor-joining.– *Cladistics*, **12**: 99-124.

FELSENSTEIN, J., 1985.– Confidence limits on phylogenies: An approach using the bootstrap.– *Evolution*, 39: 783-791.

FERNÁNDEZ-VIDAL, E. H., 1992.– Comentarios de la distribución geográfica francesa y notas sobre *Graellsia isabelae* (Graells, 1849) (Lepidoptera: Saturniidae).– *SHILAP Revta. lepid*., **20**(77): 29-49.

GOLOBOFF, P., 1993.– *NONA ver. 2*. Published by the author, Tucumán, Argentina.

GRIVEAUD, P., 1961.– Insectes, Lépidoptères Eupterotidae et Attacidae.– *Faune Madagascar,* **14**: 64 pp., 12 pls.

HEPPNER, J. B., WANG, H. Y. & CHANG, Y. C., 1988.– Larval morphology of Taiwan Saturniidae (Lepidoptera): *Actias selene ningpoana* Felder.– *J. Taiwan Mus.,* **41**(2):107-114.

HOLLOWAY, J. D. & NIELSEN, E. S., 1999.– Biogeography of the Lepidoptera, pp. 423-462. *In* N. P. KRISTENSEN, ed., Lepidoptera, Moths and Butterflies, vol. 1: Evolution, Systematics, and Biogeography, Part 35 of *Handbook of Zoology, vol. **4**: Arthropoda: Insecta*: x + 491 pp. W. de Gruyter, Berlin.

JANZEN, D. H., 1984.– Two ways to be a tropical big moth: Santa Rosa saturniids and sphingids.– *Oxf. Surv. evol. Biol*., **1**: 85-140, 4 col. pls.

JORDAN, K., 1911.– Saturniidae: pp. 209-226, pls. 31-35. *In* A. SEITZ, (ed.), *Die Großschmetterlinge der Erde*, *Die palaearktischen Spinner & Schwärmer*, **2***:* vii + 479 pp, 56 col. pls. A. Kernen Verlag, Stuttgart.

JOST, B., SCHMID, J. & WYMANN, H.–P., 2000.– Saturniidae, Pfauenspinner/ Nachtpfauenaugen, pp. 367-398, col. pls.16-19. *In*: *Schmetterlinge und ihre Lebensräume: Arten, Gefährdung, Schutz; Schweiz und angrenzende Gebiete,* **3**: xi + 914 pp. Pro Natura-Schweizerischer Bund für Naturschutz, Egg.

KISHINO, H. & HASEGAWA, M., 1989.– Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea.– *J. molec. Evol.*, **29**:170–179.

KLOTS, A. B., 1970.– Lepidoptera, pp. 115-130. *In* S. L. TUXEN, ed., *Taxonomist's Glossary of Genitalia in Insects.* Munksgaard.

LEMAIRE, C., 1978.– *Les Attacidae Americains...The Attacidae of America (= Saturniidae), Attacinae*: 238 pp., 49 pls. C. Lemaire, Neuilly-sur-Seine.

MADDISON, W. P. & MADDISON, D. R., 2002.– *MacClade 4, vers. 4.05*. Sinauer Associates, Sunderland, Massachusetts.

MATSUDA, R., 1965.– Morphology and evolution of the insect head.– *Mem. Am. ent. Inst.*, **4**: 1-334.

MATSUDA, R., 1970.– Morphology and evolution of the insect thorax.– *Mem. ent. Soc. Can.*, **76**: 1-431.

MATSUDA, R., 1976.– *Morphology and Evolution of the Insect Abdomen*: viii + 534 pp. Pergamon Press, Oxford

MELL, R., 1950.– Aus der Biologie der chinesischen *Actias* Leach .– *Entomol. Z.*, **60**(6): 41-45, (7): 53-56.

MOHANRAJ, P., VEENAKUMARI, K. & PEIGLER, R. S., 1996.– The host plant and pre- imaginal stages of *Actias callandra* (Saturniidae) from the Andaman Islands, India.– *J. Res. Lepid.*, **32**: 16-25.

MORISHITA, K. & KISHIDA, Y., 2000.– Moths in Nanling Mountains, Guangdong, S. China.– *Yadoriga*, **187**: 10-17. [in Japanese].

MOUCHA, J., 1966.– *Beautiful Moths*: 139 pp. Spring Books, London.

NÄSSIG, W., 1991.– Biological observations and taxonomic notes on *Actias isabellae* [*sic*] (Graells) (Lepidoptera: Saturniidae).– *Nota lepid.*, **14**(2):131-143.

NÄSSIG, W. A. & PEIGLER, R. S., 1984.– The life-history of *Actias maenas* (Saturniidae).– *J. Lepid. Soc.*, **38**(2): 114-123.

NAUMANN, S., 1995.– *Die Saturniiden-Fauna von Sulawesi, Indonesien*: 145 pp. Doctoral Dissertation, Freie-Universitat, Berlin.

NIXON, K. C., 2002.– *WinClada ver. 1.00.08.* Published by the author, Ithaca, New York.

PAUKSTADT, U. & PAUKSTADT, L. H., 1993.– Die Präimaginalstadien von *Actias groenendaeli* Roepke 1954 von Timor, Indonesien, sowie Angaben zur Biologie und Ökologie (Lepidoptera: Saturniidae).– *Ent. Z., Frankf. a. M.,* **103**(17): 305-314.

PEIGLER, R. S., 1978.– Hybrids between *Callosamia* and *Samia* (Saturniidae).– *J. Lepid. Soc.*, **32**(3): 191-197.

PEIGLER, R. S., 1986.– Worldwide predilection of resiniferous hostplants by three unrelated groups of moths in the genera *Actias*, *Citheronia* (Saturniidae) and subfamily Euteliinae (Noctuidae).– *Tyô to Ga*, **37**(1): 45-50.

PEIGLER, R. S. & NAUMANN, S., 2003.– *A Revision of the Silkmoth Genus Samia*: 230 pp., 10 maps, 16 pls. University of the Incarnate Word, San Antonio.

PEIGLER, R. S. & WANG, H. Y., 1996.– *Saturniid Moths of Southeastern Asia*: vi + 265 pp. Taiwan Museum, Taipei. [in Chinese and English].

PHILLIPS, R., & RIX, M., 2002.– *The Botanical Garden, vol. 1: Trees and Shrubs*: 491 pp. Firefly Books, Buffalo, New York.

PINHEY, E., 1972.– *Emperor Moths of South and South Central Africa*: xi + 150 pp., 43 pls. C. Struik (Pty) Ltd., Cape Town.

REGIER, J. C., MITTER, C., PEIGLER, R. S. & FRIEDLANDER, T. P., 2002.– Morphology, composition, and relationships within Saturniinae (Lepidoptera: Saturniidae): Evidence from two nuclear genes.– *Insect Syst. Evol.,* **33**: 9-12.

REGIER, J. C., PAUKSTADT, U., PAUKSTADT, L. H., MITTER, C., & PEIGLER, R. S., 2005.– Phylogenetics of eggshell morphogenesis in *Antheraea* (Lepidoptera: Saturniidae): unique origin and repeated reduction of the aeropyle crown.– *Syst. Biol.*, **54**(2): 1-14.

SNODGRASS, R.E., 1935.– *Principles of Insect Morphology*: ix + 667 pp. McGraw-Hill, New York.

STEHR, F. W., 1987.– *Immature Insects*: 754 pp. Kendall/Hunt, Dubuque, Iowa.

SWOFFORD, D. L., 2002.– *PAUP* Phylogenetic Analysis Using Parsimony (*and other methods). ver. 4.0b10.* Sinauer Associates, Sunderland, Massachusetts.

TEMPLETON, A. R., 1983.– Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes.– *Evolution*, **37**: 221–244.

TESTOUT, H., 1940.– Contributions à l'étude des Lépidoptères Saturnioïdes (VIII), Révision des Saturnioïdes macroures (Actiens de Sonthonnax). Présentation et remarques sur les *Argema* africains (Lép. Saturniidae).– *Bull. mens. Soc. linn. Lyon*, **9**: 82- 91.

VEENAKUMARI, K., MOHANRAJ, P. & PEIGLER, R. S., 2005.– Life history of *Actias ignescens* (Lepidoptera, Saturniidae) reared on its natural hostplant, *Crypteronia paniculata*, on South Andaman Island.– *Trans. Lepid. Soc. Japan*, **56**(3): 184-192.

YLLA, J., 1995.– Efectes del diflubenzuró sobre una població de *Graellsia isabelae* (Graells, 1849) (Lepidoptera: Saturniidae).– *Treb. Soc. Cat. Lep*., **12**(1993-1994): 5-12.

YLLA, J., 1997.– *Història Natural del Lepidòpter Graellsia isabelae* (Graells, 1849): 232 pp., 27 col. figs. Institut d'Estudis Catalans, Barcelona.

YLLA, J. & SARTO, V., 1993.– Ecological factors affecting mating of *Graellsia isabelae* Graells, 1849) (Lepidoptera: Saturniidae).– *Nota lepid*., **16**(2): 145-162.

ZHU, H. F. & WANG, L. Y., 1996.– Lepidoptera: Bombycidae, Saturniidae, Thyrididae.– *Fauna Sinica, Insecta,* **5**: 302 pp., 18 pls. [in Chinese].

J. Y.
Urbanització Serrabonica
Gurb (Osona)
E-08503 Barcelona
ESPAÑA / *SPAIN*

R. S. P.
Department of Biology
University of the Incarnate Word
4301 Broadway
San Antonio, Texas 78209-6397
EE.UU. / *U.S.A.*

A. Y. K.
Maryland Center for Systematic Entomology
University of Maryland
4112 Plant Sciences Building
College Park, Maryland 20742-4454
EE.UU. / *U.S.A.*

(Recibido para publicación / *Received for publication* 9-VI-2005)
(Revisado y aceptado / *Revised and accepted* 10-VII-2005)

Figs. 1-6.– Adults and mature larvae of *Actias*. **1.** *Actias truncatipennis*, female from Las Minas, Veracruz, Mexico. **2.** *Actias maenas*, male from Tapah, Perak, Malaysia. **3.** *Actias dubernardi*, male from Dahongshan, 1600 m, Shuizhou, Hubei Province, China. **4.** *Actias sinensis*, male from Jianlongshan, 1500 m, Hunan Province, China. **5.** *Actias dubernardi* on *Pinus eldarica*. **6.** *Actias sinensis* on *Liquidambar formosana*. All specimens were reared from eggs and photographed by R. S. Peigler. Specimens (Figs. 1-4) were deposited in Department of Entomology, Texas A&M University.

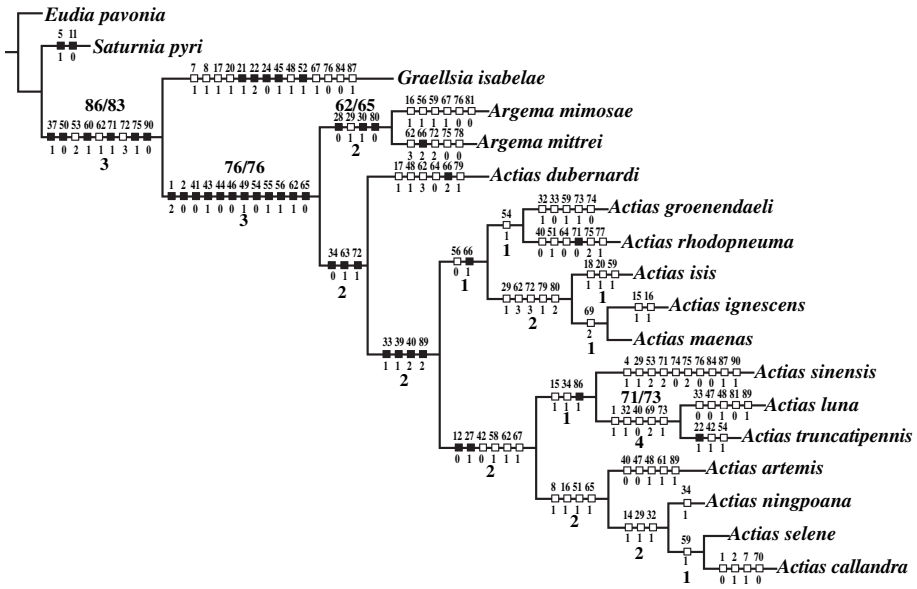


Fig. 7.– The most parsimonious tree (L = 225, CI = 0.52, RI = 0.61) obtained from the morphological phylogenetic analysis under unambiguous optimization. Filled squares indicate synapomorphies, white squares delineate homoplasies. Small numbers above each square are character numbers, and character transformations present in derived lineages are shown below each square. Large numbers below each branch denote Bremer support values, and large numbers above each branch indicate bootstrap/jackknife (> 50%) values, respectively.

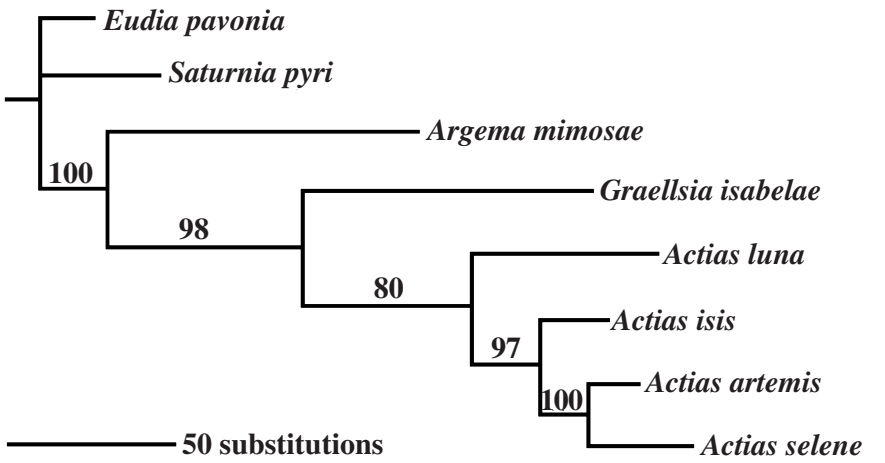


Fig. 8.– The most parsimonious tree (L = 402, CI = 0.88, RI = 0.71) generated from the molecular analysis of six ingroup species and two outgroups. Bootstrap values (> 50%) are indicated above each branch.

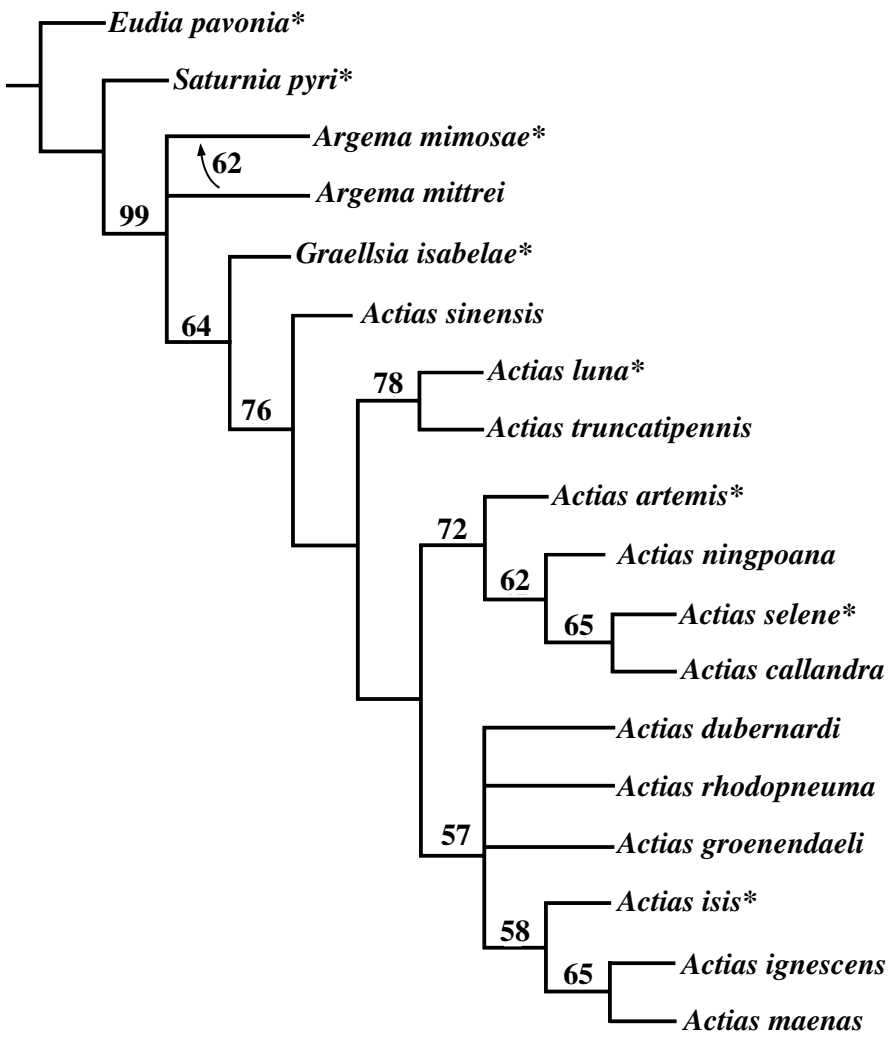


Fig. 9.– Strict consensus of six most parsimonious trees (L = 636, CI = 0.74, RI = 0.62) produced from the combined analysis (morphology + behaviour + EF1-" + DDC). The curved arrow indicates a sister-group relationship that was not recovered in the strict consensus, but a relationship that was present in the BP analysis. Asterisks denote taxa for which molecular data were analyzed.

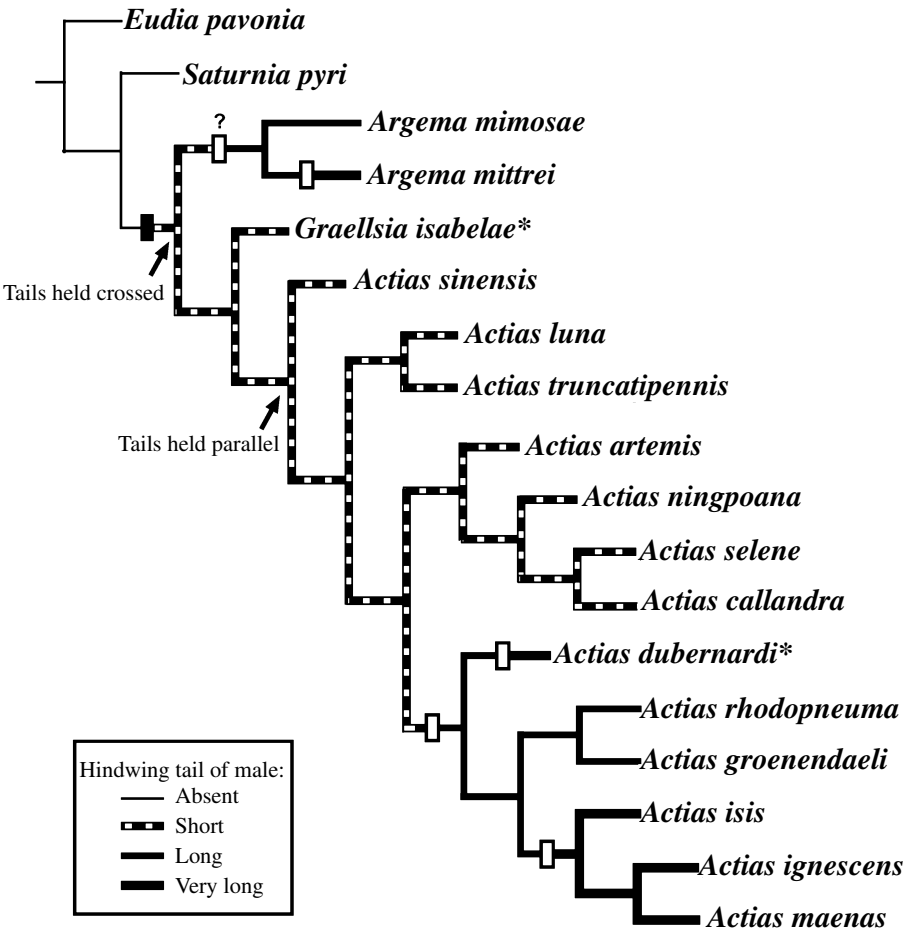
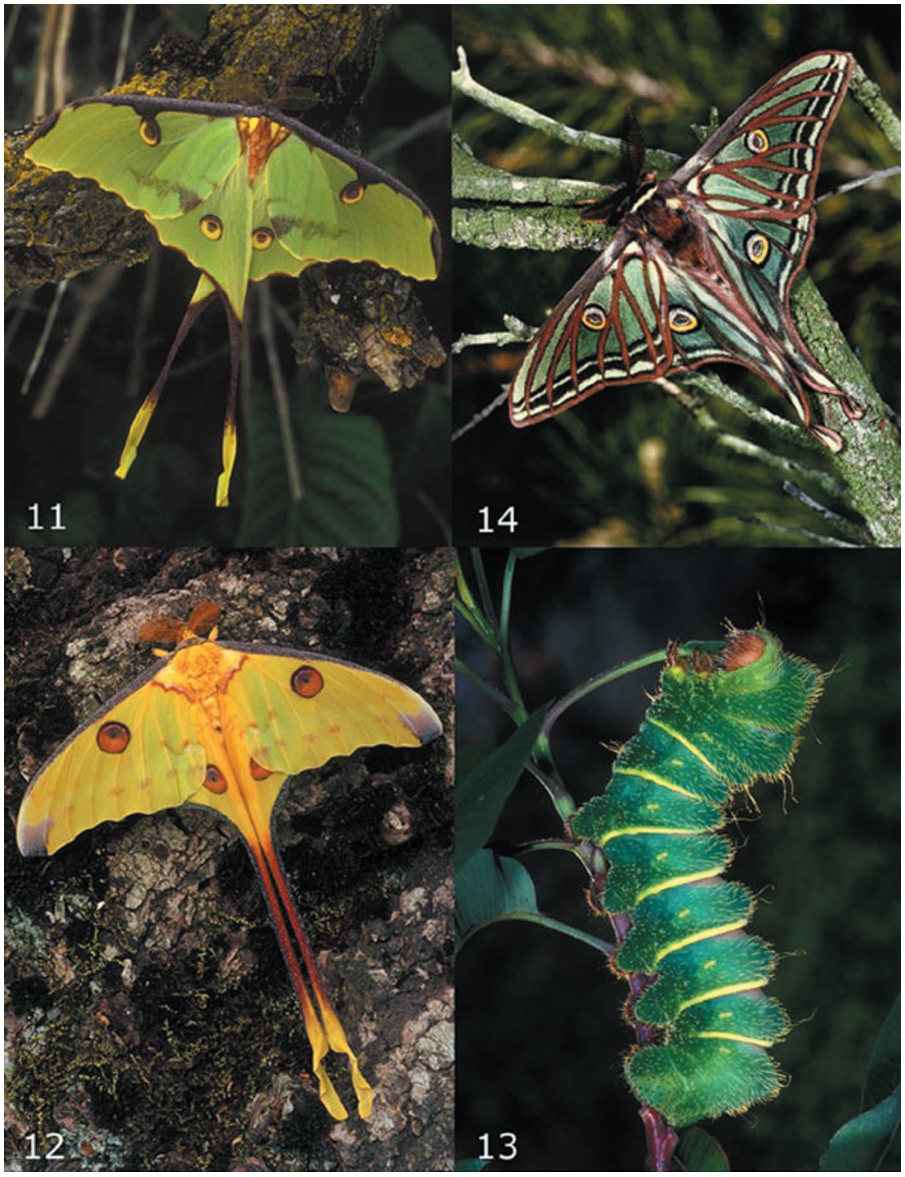


Fig. 10.– Evolution of the male hindwing tail and larval hostplant feeding in moon moths. Character-state changes were examined under unambiguous optimization. Our results indicate that tail length evolved once from no tail to short, and then lengthened multiple times. Three lineages independently evolved very long tails. The posture at which hindwing tails are held evolved from an ancestral state at which tails were crossed before tails were held parallel. The black rectangle indicates an origin for the short tail length (synapomorphy), white rectangles indicate homoplasies. The question mark indicates an uncertain homoplasy which is not present in all six trees (see Fig. 9). Asterisks after taxon names denote taxa with larvae that feed on conifers.



Figs. 11-14.– 11. *Argema mimosae*, male (southern Zaire); **12.** *Argema mittrei*, male (Madagascar); **13.** *Argema mittrei*, 5th instar larva; **14.** *Graellsia isabelae*, male (Spain). Photographs courtesy of Kirby L. Wolfe.